SOP: SP042

Electroporation of *M. smegmatis*

Materials and Reagents:

1. Frozen or freshly made 400µl aliquot of electrocompetent *M. smegmatis*
2. Ice in ice bucket
3. Pipettman, 1000µl, 200µl, 10µl
4. Sterile pipette tips, 1000µl, 200µl, 10µl
5. GenePulser electroporation cuvette with a 0.1 cm electrode gap (Invitrogen Catalog no. 65-0030)
6. GenePulser electroporator (Bio-Rad catalog no. 1652660 through 1652668)
7. Kimwipes
8. Sterile LB broth
9. 15ml conical Falcon tubes (VWR catalog no. 21008-918)
10. 37°C shaking incubator
11. LB plates with appropriate antibiotics
12. 37°C incubator
13. Plastic bags
14. Allegra 6 R tabletop centrifuge
15. Autoclave bags
16. Autoclave tape
17. Autoclave

Protocol:

1. _____ Thaw a 400µl aliquot of electrocompetent *M. smegmatis* on ice.

2. _____ Once thawed, swirl transforming DNA (0.1µg) into the cells and incubate on ice for 5 min.

3. _____ Transfer cells and DNA to a pre-chilled GenePulser electroporation cuvette with a 0.1-cm electrode gap (Invitrogen Catalog #65-0030) and rap smartly against the benchtop in order to draw all cells to the bottom. Return to ice.

4. _____ Flip the power switch on the right side of the GenePulser electroporator to "ON". A menu will appear.

5. _____ Choose "#1 Exponential Protocol" by pushing "Enter".

6. _____ Fill in 1.25kV, 25µF (capacitance), 1000Ω (resistance), 1mm (electrode gap size) by navigating the menu with the arrow buttons. Once finished push "Enter". A "P" will begin to flash in the bottom right corner of the screen. This indicates that the machine is ready to pulse.

7. _____ Cap the cuvette and wipe all moisture from the outside of the cuvette. Any remaining moisture could cause the machine to arc.

8. _____ Place the cuvette into the safety chamber of the electroporator. Orient the cuvette so that the metal plates are aligned with the electrodes. Push the cuvette into the chamber until it is sealed between the electrodes and the base of the chamber. Close the lid on the safety chamber.

9. _____ Press and hold the large red "Pulse" button until the machine beeps and the screen changes to show your exponential curve.

10. _____ Record the time constant and voltage applied. A time constant of 1.0-10s is optimal. A voltage of 1000-1500Ω is optimal.

11. _____ Immediately transfer the cuvette to ice and gently resuspend the cells in 1 ml of sterile LB. The number of transformants declines as the cells remain without media.

12. _____ Transfer the cells and media to a sterile 15ml conical Falcon tube (VWR catalog no. 21008-918). Loosen the cap on the tube to allow proper aeration and secure with tape to prevent it being shaken off.

13. _____ Place the tube in the 37°C shaking incubator for 4 hours. This allows the cells to begin expressing antibiotic resistance genes.

14. _____ Warm two LB-antibiotic plates to room temperature.

15. _____ Spin down the cells in an Allegra 6 R tabletop centrifuge for 10 min at 3000 rpm, 4˚C. Pipette off supernatant. Resuspend cells in 500 µl sterile LB.

16. _____ Plate 50µl of resuspension on one of the plates and 100µl on the other. Allow plates to dry, invert, and place in plastic bag. Place this in the 37°C incubator. Flat, crinkled, white colonies should appear in 3-4 days.

17. _____ Autoclave and discard all wastes generated, including the electroporation cuvette.